Nokireki *et al. Acta Vet Scand (2017) 59:62*
DOI 10.1186/s13028-017-0331-y

Acta Veterinaria Scandinavica

BRIEF COMMUNICATION Open Access

Second case of European bat lyssavirus type 2 detected in a Daubenton's bat in Finland

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Abstract

European bat lyssavirus type 2 (EBLV-2) was detected in Finland in a Daubenton's bat (*Myotis daubentonii*) found in the municipality of Inkoo (60°02′45″N, 024°00′20″E). The bat showed neurological signs and was later found dead. The laboratory analysis revealed the presence of lyssavirus, and the virus was characterized as EBLV-2. This isolation of EBLV-2 was the second time that the virus has been detected in a Daubenton's bat in Finland. This provides additional proof that EBLV-2 is endemic in the Finnish Daubenton's bat population.

Keywords: Daubenton's bat, EBLV-2, European bat lyssavirus type-2, Finland, *Myotis daubentonii*

Findings

Rabies is a fatal encephalomyelitis caused by lyssaviruses with a case fatality rate of almost 100%. Rabies virus (RABV) causes about 99% of all rabies cases in humans, mostly in Asia and Africa. Thirteen other lyssavirus species have been accepted by the International Committee on Taxonomy of Viruses [1] and two additional species have been identified Lleida bat lyssavirus from Spain in 2011 [2] and Gannoruwa Bat Lyssavirus from Sri Lanka in 2015 [3]. Bats are considered to be the true reservoir of lyssaviruses [4]. There is evidence that bats seroconvert after exposure to lyssaviruses without development of clinical signs, but in some cases, bats develop clinical disease similar to rabies in other mammals and death occurs after the appearance of clinical signs [5]. Finland has been free of RABV since 1991, but European bat lyssavirus type 2 (EBLV-2) was detected in 2009 in a Daubenton's bat (*Myotis daubentonii*) [6]. In addition, antibodies against lyssavirus have been detected in Daubenton's bats from the same area [7]. EBLV-2 has sporadically been isolated from bats in the Netherlands [8], Switzerland [9], the United Kingdom [10], Finland [6], Germany [11] and Norway [12]. EBLV-2 has caused two human cases: in Finland in 1985 [13] and in the United Kingdom in 2002 [14]. Both victims were researchers studying bats and they did not receive pre- or post-exposure rabies prophylaxis. No spill-over infections to other mammals than humans have been detected for EBLV-2.

A private citizen observed an abnormally behaving bat at a summer cottage in the municipality of Inkoo (60°02′45″N, 024°00′20″E) in October, 2016. Inkoo is in the province of southern Finland and is part of the Uusimaa region. The bat exhibited behavioral changes: it appeared during daytime, was unable to crawl into the roof space of a building, and had difficulties in moving and flying. When crawling on the wall, the bat had severe ataxia and tetraparesis. Later that day, the bat was grounded and found dead. It was sent to the Finnish Food Safety Authority Evira for autopsy. The Daubenton's bat was a cachexic adult female, weighing circa 7 g (Fig. 1).

The presence of lyssavirus was detected in the brain by fluorescent antibody test (FAT) [15]. Smears prepared from a sample of brain tissue were fixed in high-grade cold acetone, air dried, and then stained with specific conjugate (FITC Anti-Rabies Monoclonal Globulin, Fujirebio Diagnostics and Rabies Antinucleocapsid conjugate, Bio-Rad). FAT slides were examined for specific fluorescence using a fluorescence microscope with positive results. The brain suspension of the bat was inoculated in mouse neuroblast cells Neuro-2a (ATCC® CCL-131™) according to a rabies tissue culture infectious test (RTCIT) procedure described in the OIE manual [15], and the virus isolation for lyssavirus was positive. Additional organ and swab samples were collected from

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Fig. 1 The Daubenton's bat found dead in the municipality of Inkoo, SW Finland. Courtesy of Riitta Räisänen

the bat. RNA was extracted from the organ and swab suspensions of the bat with the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The OneStep RT-PCR kit (Qiagen) was used to amplify two fragments. The reaction volume was 25 µL and the temperature profile of cDNA synthesis and amplification was 30 min at 50 °C, 15 min at 94 °C for reverse transcriptase inactivation and DNA polymerase activation, followed by 30 amplification cycles of 1 min at 94 °C, 1 min at 50 °C, and 1 min at 72 °C. Primers were published by Davis et al. [16]. The results are presented in Table 1. After agarose gel electrophoresis, the band of the brain sample was cut from the gel and DNA was extracted with the Qiaquick Gel Extraction Kit (Qiagen). The reaction products were purified using the DyeEx 2.0 Spin Kit (Qiagen). PCR products were sequenced using an ABI 3100 Avant Genetic Analyzer (Applied Biosystems) with the primers used in the PCR and a Big Dye Terminator v3.1 Cycle sequencing kit (Applied Biosystems). The sequences were analyzed with DNASTAR Lasergene 10.

There have been two cases of EBLV-2 from Daubenton's bats in Finland, the first in 2009 [6] and now in 2016. This provides further evidence that EBLV-2 is enzootic in Daubenton's bats in Finland, at least in the southwestern part of the country. However, we consider the risk of EBLV-2 infection of humans as extremely low. Adequate information should be given to the general public on what to do when they come into contact with bats. People who handle bats due to their work or hobby should be vaccinated against rabies according to the guidelines of WHO [17].

Even though Daubenton's bats are most likely the true reservoir of EBLV-2, they can become diseased and show typical neurological signs of rabies: abnormal behavior, paralysis, and coma followed by death. Therefore, passive surveillance of sick and dead bats is the most important surveillance method. Researchers studying bats and members of the public play a key role in providing samples to the diagnostics laboratory. Active surveillance of healthy bats has seldom revealed lyssaviruses from bats [7, 18].

FAT has been proved to be effective in detecting EBLV-2 from infected bats, but in one recorded case, the FAT test was negative on a bat sample even though viral RNA was detected by RT-PCR and the virus was isolated in a cell culture. Some laboratories have had difficulties to reliably detect EBLV strains when using the FAT, with results depending on the rabies virus antibody conjugate and even the batch used [12].

The viral RNA was detected by RT-PCR and the viable virus was isolated using mouse neuroblast cells from the brain, the spinal cord and the salivary glands, but not from other organ or swab samples (Table 1). The virus was identified as EBLV-2 based on partial N-gene sequencing and phylogenetic analysis (Fig. 2). The sequence (GenBank Accession Number MF326269) was 98% identical to previously found EBLV-2 strains in Finland. The isolated EBLV-2 was also phylogenetically very similar to the strains characterized in other parts of Europe [19]. Characterization of the isolated lyssaviruses provides valuable information on the epidemiological situation of the reservoir of specific lyssavirus and possible spill-over species.

Table 1 Results of lyssavirus detection from different organ and swab samples from the Daubenton's bat

	Brain	Spinal cord	Salivary gland	Mouth swab	Anal swab	Tongue	Intestines	Liver	Eye	Lung	Trachea	Bladder	Tonsils
FAT	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
RTCIT	+	+	+	−	−	−	−	−	−	−	−	−	−
RT-PCR	+	+	+	−	−	−	−	−	−	−	−	−	−

FAT fluorescent antibody test, *RTCIT* rabies tissue culture infectious test, *NA* not applicable

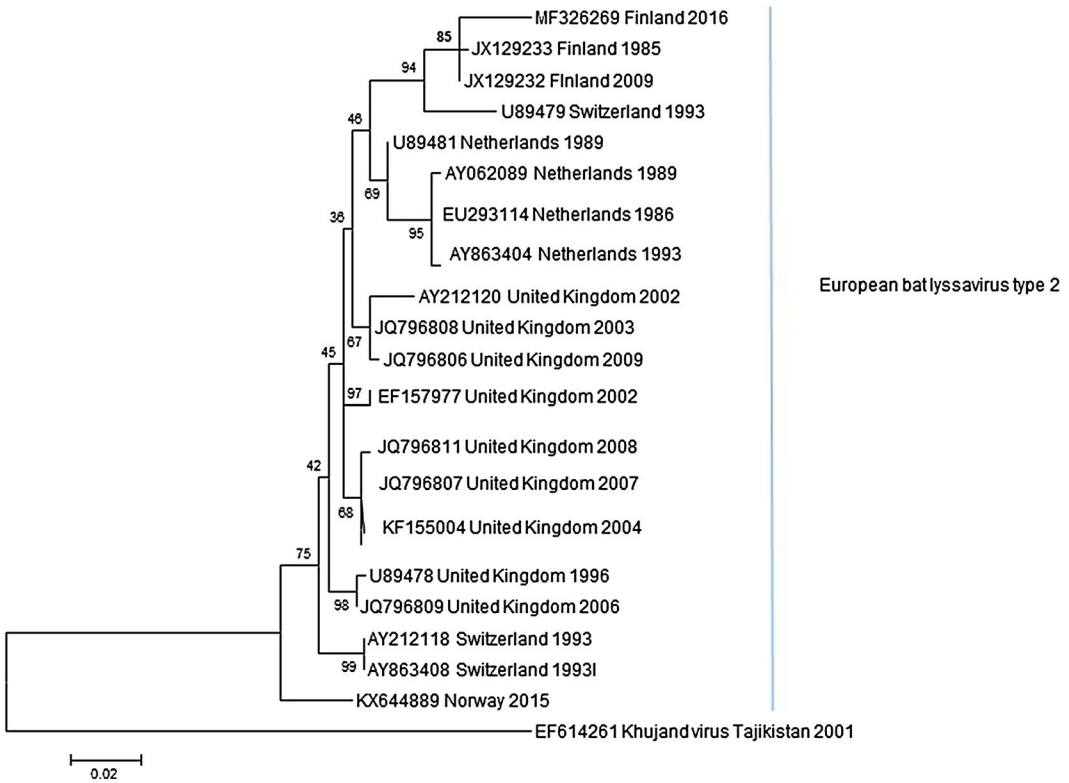

Fig. 2 A phylogenetic tree based on partial N-gene sequences. The phylogenetic tree was estimated using the maximum likelihood approach in the program MEGA with 1000 bootstrap replicates

Abbreviations

EBLV-2: European bat lyssavirus type 2; FAT: fluorescent antibody test; OIE: World Organisation for Animal Health; RABV: rabies virus; RTCIT: rabies tissue culture infectious test; WHO: World Health Organization.

Authors' contributions

TN was responsible for antigen detection, virus isolation, RT-PCR analysis, diagnostic sequencing and drafting the manuscript, TS and TSm further characterized the virus and made phylogenetic analysis, VK was responsible for the sampling of organs and swabs from the bat, LS and TG provided critical intellectual contribution. All authors read and approved the final manuscript.

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Acknowledgements

The authors would like to thank the person who send the bat to the laboratory and provided photos of the bat and Evira's Oulu laboratory for the initial autopsy.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Funding

This study was funded by the Finnish Food Safety Authority Evira.

Publisher's Note

Received: 20 June 2017 Accepted: 19 September 2017
Published online: 25 September 2017

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